Cell Stem Cell
Review

Mesenchymal Stromal Cells: Facilitators of Successful Transplantation?

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Mesenchymal stromal/stem cells (MSCs) possess immunomodulatory and reparative properties. Through specific interactions with immune cells that participate in both innate and adaptive responses, MSCs exposed to an inflammatory microenvironment can downregulate many immune effector functions. Clinical trials focusing on MSCs to treat graft-versus-host disease (GvHD) and autoimmune diseases are underway. Current analyses suggest that MSCs will improve cell and solid organ transplantation by ameliorating rejection and possibly eliminating the requirement for prolonged regimens of conventional immunosuppressive drugs. This review examines the in vitro and in vivo evidence for the clinical use of bone marrow derived MSCs.

Transplantation is a life-preserving therapy for many patients with established organ failure. The development of immunosuppressive drugs has made cell and solid organ transplantation a viable therapeutic option, as rejection of foreign tissues can be delayed or prevented. However, long term administration of nonspecific immunosuppression has detrimental consequences including increased susceptibility to infection, a higher risk of tumorigenesis, cardiovascular complications, de novo induction of diabetes and renal failure (López et al., 2006). Therefore, the development of an alternative immunosuppressive therapy that is both specific and non-toxic is essential with regard to improving the long term outcome for transplant patients. A growing body of evidence suggests that a multipotent population of mesenchymal stromal cells (MSCs), also called mesenchymal stem cells, may fill this need due to their capacity to modulate immune responses via a host of direct and indirect interactions with a broad range of cell types. In this review, we discuss the results of in vitro and in vivo experiments that examine the mechanistic interactions of bone marrow stromal cells (BMSCs) with components of the immune system. Early stage clinical application of these cells is underway, despite considerable variability in preclinical experimental findings, and while initial results appear promising, there remain many open questions as to how BMSCs may function in a setting of therapeutic transplantation.

An Introduction to MSCs

In the 1960s and 1970s, Friedenstein and colleagues described the presence of stromal cells and bone forming cells within the bone marrow (Friedenstein et al., 1976). This work demonstrated that a subpopulation of bone marrow cells displayed osteogenic potential and were characterized by their rapid adherence to tissue culture plastic, fibroblast-like appearance, and their colony forming unit (CFU) capacity. Transplantation of bone marrow cells beneath the kidney capsule or in the subcutaneous space demonstrated the capacity of bone marrow cells to form ectopic marrow, consisting of trabecular bone, adipocytes, and myelosupportive stroma (Friedenstein et al., 1966, 1974; Owen, 1988). Subsequently, in the late 1980s, Owen and Caplan elaborated on this early work and proposed the idea of a nonhematopoietic adult stem cell in the bone marrow (Caplan, 1991; Owen, 1988). The term mesenchymal stem cell was coined by Caplan in 1991 (Caplan, 1991). Human bone marrow-derived MSCs were first isolated by Caplan's group (Haynesworth et al., 1992b), and the same group identified the first MSC expressed antigens that react with SH-2 (CD105) and SH-3 (CD73) (Haynesworth et al., 1992a). Since then, MSCs have been isolated from a number of other sources, including umbilical cord blood, adipose tissue, muscle, and liver (da Silva Meirelles et al., 2006; Kern et al., 2006).

Initially, MSCs were thought to mediate tissue and organ repair by virtue of a multilineage differentiation potential that enabled them to replace damaged cells (Mahmood et al., 2003; Murphy et al., 2003). However, subsequent findings suggest that this mechanism is unlikely (Caplan and Dennis, 2006; Prockop, 2009). It is now widely believed that in response to tissue injury, MSCs home to the site of damage and encourage repair through the production of trophic factors, including growth factors, cytokines, and antioxidants (Block et al., 2009; Chen et al., 2008; Karp and Leng Teo, 2009), some of which provide the basis for their capacity to modulate immune responses.

MSC Phenotype, Characteristics, and Culture

While a broad, promiscuous differentiation potential sometimes ascribed to MSCs remains debated in the literature, a subset of MSCs has been shown to contain a multipotent stem cell (Bianco et al., 2008) with the ability to differentiate into adipose tissue, bone, and cartilage (Pittenger et al., 1999). In the absence of a marker specific for these multipotent MSCs, a panel of markers has been outlined by the International Society for Cellular Therapy (ISCT) and widely adopted by the field to phenotypically define the population. The ISCT has stated that "MSCs must express CD105, CD73 and CD90 and lack the expression of CD45, CD34, CD14, or CD11b, CD79α or CD19 and HLA-DR surface molecules" (Dominici et al., 2006). Furthermore, MSCs are selected in vitro by adherence to tissue-culture-treated plastic. Significant advances have been achieved over the last

few years with regard to unifying the identification and culture of human MSC populations in particular. However, it is important to add that the above criteria serve as a means to characterize the cells but that the combined surface phenotype is not definitive. It is likely that this definition will continue to evolve over time.

It should also be noted that multiple differences between mouse and human MSCs have been reported. Mouse MSCs have not yet been formally characterized with respect to surface antigen expression but generally follow the human MSC criteria. While it is relatively easy to isolate and expand human MSCs, mouse MSC cultures can be highly contaminated with hematopoietic cells, even after multiple passages, and this heterogeneity likely contributes to the conflicting results produced by different laboratories, each utilizing different populations of "MSCs."

MSCs can be passaged in vitro through many rounds of culture, yet there are indications that chromosomal modifications, replicative senescence, and loss of function can occur as a result of prolonged ex vivo culture (Briquet et al., 2010; Miura et al., 2006; Wagner et al., 2008), and therefore, the consensus is that MSCs should be used for cell therapy only at low passages. Variations in in vitro culture conditions can lead to differences in MSC function. In particular, MSCs cultured under hypoxic conditions (low oxygen) display enhanced tissue regeneration potential in mouse models of myocardial infarction, via elevated production of trophic factors (Hu et al., 2008; Rosová et al., 2008). While in the majority of studies MSCs are cultured under normoxic conditions, it is important that individual reports highlight the specific conditions used in order to understand differences between laboratories and to determine the optimal conditions for MSC culture to enable experimental data to be relevant for clinical studies.

A better understanding of the in vivo origin of MSCs has been eagerly awaited since the realization that MSCs held significant reparative and immune modulatory potential. Progress toward this goal has emerged over the past 2 years, with data suggesting that MSCs arise from the perivascular zone and that they can function as vascular pericytes (Crisan et al., 2008). However, while MSCs share many similarities with pericytes, there are subtle differences that distinguish these populations, and therefore, further data are required to substantiate this hypothesis.

Interestingly, it appears that fibroblasts share many of the same characteristics as MSCs, including immunosuppressive effects, differentiation potential, plastic adherence, and surface marker expression in vitro (reviewed in Haniffa et al., 2009). Some evidence exists to suggest that both MSC and fibroblasts display trilineage differentiation potential at the clonal level (Chen et al., 2007; Muraglia et al., 2000). Importantly, the frequency of MSCs appears to be very low in vivo, making them rare in comparison to fibroblasts. The high risk of "mistaken identity" between these populations highlights that the development of assays that can distinguish MSCs from similar cells should be a high priority for the field.

To date, there has been much ambiguity with regard to the correct nomenclature used to describe this population of cells, which are referred to as mesenchymal stem cells by some authors and mesenchymal stromal cells by others (Bianco et al., 2008). Because bone marrow stromal cells (BMSCs) are the best characterized population, we focus our review on these cells.

BMSC Immune Modulation

The initial studies carried out by Friedenstein and colleagues in the 1960s and 1970s and the subsequent work by Caplan and Owen demonstrated the existence of a subpopulation of multipotent cells within the bone marrow that exhibited the capacity to support hematopoiesis (Caplan, 1991; Friedenstein et al., 1966; Friedenstein et al., 1970; Friedenstein et al., 1974; Owen, 1988). An array of studies attempted to harness the hematopoiesis support property of BMSCs to facilitate or enhance hematopoietic stem cell (HSC) engraftment. In 1995, autologous, culture-expanded BMSCs were given to patients with hematological malignancies that were in complete remission and demonstrated safety with no reports of adverse events (Lazarus et al., 1995). Subsequently, a phase I/II trial in patients receiving myeloablative therapy for breast cancer demonstrated the capacity of autologous BMSCs to enhance HSC engraftment (Koç et al., 2002).

Bartholomew and colleagues were one of the first groups to extend the study of how BMSCs impact the formation and engraftment of blood cells to how they influence the function of mature immune cells. They did so by demonstrating that BMSCs are immunosuppressive in vitro and in vivo using a baboon skin allograft model (Bartholomew et al., 2002). Di Nicola et al. (2002) went a step further by identifying that soluble factors are important in BMSC-mediated immune modulation. Furthermore, a large body of in vitro data supported these early studies by demonstrating an immunosuppressive role for BMSCs via suppression of T cell proliferation (Aggarwal and Pittenger, 2005; Glennie et al., 2005; Klyushnenkova et al., 2005; Krampera et al., 2003; Potian et al., 2003; Tse et al., 2003) or through modulation of antigen-presenting cell phenotype and function (Beyth et al., 2005; Groh et al., 2005; Jiang et al., 2005; Zhang et al., 2009; Zhang et al., 2004). Armed with the knowledge that BMSCs displayed immunosuppressive properties, two independent groups used ex vivo expanded BMSCs to treat patients with steroid refractory graft-versus-host disease (GvHD) in small scale pilot studies (Lazarus et al., 2005; Le Blanc et al., 2004) with promising results. Furthermore, BMSCs are currently in trials for use in Crohn's disease, type 1 diabetes mellitus, and multiple sclerosis (www.clinicaltrials.gov) (Ankrum and Karp, 2010). While preliminary results look encouraging, there is currently a distinct lack of mechanistic data with regard to how BMSCs regulate immune cells in vivo. Experimental progress is being made toward filling in these gaps in our understanding, as discussed below.

Transplantation Rejection and the Impact of BMSCs

The paramount goal in transplantation is the acquisition of a state of tolerance or immunological unresponsiveness, and evidence is mounting to suggest that BMSCs may facilitate this outcome. In addition to their ability to produce trophic factors, BMSCs also exhibit potent anti-inflammatory effects both in vitro and in vivo. In this fashion, BMSCs have the capacity to regulate the activity of T cells, B cells, DCs, natural killer cells (NKs), and macrophages (Asari et al., 2009; Németh et al., 2009; Sheng et al., 2008; Spaggiari et al., 2008) either directly through cell:cell contact or indirectly via the production of soluble factors. Alongside these cellular interactions, the homing of BMSCs to sites of inflammatory insult and subsequent activation of

immunomodulatory mechanisms in such a microenvironment suggest that BMSCs have the potential to regulate the immune response in a highly specific manner (Ding et al., 2010; Ren et al., 2008).

Interactions between BMSCs and T Cells

Transplant rejection is typically mediated by both T cell-mediated and humoral- or antibody-mediated immune responses (Colvin and Smith, 2005; Hall et al., 1978). The induction of T cell proliferation and activation of effector functions is the key driving force in the majority of rejection cases (Hall et al., 1978). Following solid organ transplantation, alloantigen can be presented to naive and memory T cells via donor or host antigen-presenting cells (APCs) (LaRosa et al., 2007; Shoskes and Wood, 1994) expressing major histocompatibility (MHC) molecules. In response to alloantigen stimulation, T cells are activated, proliferate, and differentiate into effector cells. Different subsets of effector T cells are defined by their precise properties, and induction of a specific effector subset is determined by the microenvironment in which the activation takes place (Heidt et al., 2010; Murphy and Stockinger, 2010).

BMSC Modulation of T Cells

BMSCs have been shown repeatedly to suppress T cell proliferation in vitro, whether mitogen or alloantigen driven (Di Nicola et al., 2002; Ding et al., 2009; English et al., 2007; Glennie et al., 2005), but, notably, have little effect on virally driven T cell proliferation (Karlsson et al., 2008). This suppression is not thought to be MHC restricted (Comoli et al., 2008; Le Blanc et al., 2008). The effect of BMSCs on T cell proliferation appears to be dependent on the inflammatory environment present when the two cell types interact (Najar et al., 2009; Polchert et al., 2008; Ren et al., 2008). For example, BMSC mediated inhibition of T cell proliferation occurs under proinflammatory, but not anti-inflammatory, conditions (Comoli et al., 2008). These observations correlate with mechanistic studies that identified a requirement for proinflammatory cytokines (IFN-γ, TNF-α, and/or IL-1β) to elicit BMSC activation (Ren et al., 2008). An in vivo GvHD model further substantiated such views in that IFN-γ was demonstrated to be necessary for BMSCs to suppress disease development (Polchert et al., 2008).

Coculture with BMSCs also modifies the ratio of $CD4^+$ T cell subsets. In particular, a skewing that favors an increase in regulatory T cells (Tregs) and Th1 cells that accompanies a corresponding decrease in Th2 and Th17 cells is typically observed (Casiraghi et al., 2008; Ge et al., 2009; Kong et al., 2009; Rafei et al., 2009). This augmentation of T cell subsets was demonstrated in semi- and fully allogeneic mouse cardiac allograft transplantation models, a mouse model of experimental autoimmune encaphelomyelitis, as well as a rat model of experimental autoimmune myasthenia gravis (Casiraghi et al., 2008; Ge et al., 2009; Kong et al., 2009; Rafei et al., 2009).

The increase in the frequency of Tregs after coculture with BMSCs is significant in a transplantation setting, as Tregs typically maintain tolerance to self-antigens and assist in the prevention of autoimmunity. Tregs can also control alloreactive T cell responses (Long and Wood, 2009; Nadig et al., 2010; Wood and Sakaguchi, 2003). In vivo, BMSC-induced Tregs were demonstrated to be donor specific (Casiraghi et al., 2008). Mechanistically, the factors required for the BMSC driven generation of Tregs (analyzed by an increase in expression of the transcription factor FoxP3 and the cell surface marker CD25, IL-2rα chain) include PGE_2, TGF-β, and cell-cell contact, acting within a nonredundant capacity (English et al., 2009).

BMSCs have been demonstrated to inhibit the proliferation of the $CD8^+$ T cell subpopulation. Some studies have also shown suppression of $CD8^+$ T cell-mediated cytotoxicity (Rasmusson et al., 2007), but other reports conflict with these findings (Ramasamy et al., 2008). This discrepancy can perhaps be explained by an actual effect of BMSCs on total T cell number, via an impact on cell death. That is, if the number of $CD8^+$ T cells is decreased during coculture with BMSCs, the overall cytotoxic effect of the remaining population will be reduced. Meanwhile, the reciprocal relationship does not appear to exist, since BMSCs do not appear to be targeted for destruction by effector $CD8^+$ T cells (Rasmusson et al., 2007).

Additionally, unconventional T cells, such as invariant natural killer T (iNKT) cells and $\gamma\delta$T cells, appear to be regulated by BMSCs in a similar way to conventional T cells, in that coculture results in the inhibition of proliferation (Prigione et al., 2009). While BMSCs induced the activation of iNKTs, a decrease in the production of IFN-γ was observed. In this particular study BMSCs were identified as a target of $\gamma\delta$ T cell cytotoxicity (Prigione et al., 2009).

Mechanisms Involved in BMSC Modulation of T Cells

The induction of T cell chemotaxis (via CXCR3 upregulation) also appears to be important for BMSC-mediated antiproliferative effects (Ren et al., 2009). This finding suggests that soluble factors with a limited diffusion distance (or cell-cell contact) are required. Nitric oxide (NO) has been demonstrated to act in this manner. In response to IFN-γ and either IL-1α, IL-1β, or TNF-α, BMSCs upregulate expression of inducible nitric oxide synthase (iNOS), thus generating an increase in the production of NO (Ren et al., 2008). This upregulation of iNOS could occur in response to an increase in the transcription factor C/EBPβ and probably also requires STAT-1 activation (Xu et al., 2009). One possible mechanism by which NO could act in this capacity is through the inhibition of Stat5 phosphorylation. The chemical suppression of NOS reverses both the inhibition of Stat5 phosphorylation and T cell proliferation (Sato et al., 2007). This specific combination of proinflammatory cytokines also stimulates the release of chemokines from BMSCs (Ren et al., 2008).

While the experimental evidence from the mouse IFN-γ/NO model is strong, human BMSCs do not suppress T cell proliferation via NO production. Alternatively, it would appear that IDO may be a key factor for human BMSCs (Ren et al., 2009). IFN-γ stimulation upregulates IDO production in human BMSCs (Ryan et al., 2007), thus suppressing T cell proliferation either by depleting tryptophan (an essential amino acid) in the local microenvironment or by causing an increase in kynurenine metabolites (Ren et al., 2009). However, data from other labs fail to demonstrate a role for IDO in human BMSC modulation of T cell proliferation but instead identify a role for HLA-G5 and IL-10 in a cell-contact-dependent manner (Selmani et al., 2008). Thus, the precise mechanisms in play still require further elucidation.

In addition to the upregulation of CXCR3, BMSCs also influence T cell chemotaxis by inducing an increase in CD62L and CCR7 on the T cell surface. This change results in T cell homing to the secondary lymphoid organs where the T cells are subsequently trapped (Li et al., 2008a).

Among the cocktail of factors secreted by BMSCs are matrix metalloproteinases (MMPs) and membrane type (MT) MMPs. BMSC have been shown to be capable of producing MMP-1, -2, -3, and -9, as well as MT1-MMP and MT3-MMP (Ding et al., 2009; Lu et al., 2010; Rafei et al., 2008). MMPs secreted by BMSC are thought to cleave CCL2 to generate an antagonistic molecule. The truncated CCL2 inhibits Th17 activation (in an experimental model of multiple sclerosis) via the indirect inhibition of STAT3 phosphorylation or, alternatively, by recruiting a STAT3 specific phosphatase (Rafei et al., 2009). Further research is required on this subject as CCR2 is not restricted to Th17 or even Th1 cells; therefore, the possible effects of antagonistic CCL2 on other T cell subsets must be established. In another study, the production of MMP-2 and -9 by BMSCs was correlated with a decrease in CD25 expression on $CD4^+$ T cells and the inhibition of alloantigen driven proliferation (Ding et al., 2009). Suppression of T cell proliferation could be due to the induction of a state of IL-2 unresponsiveness (due to loss of the IL-2rα chain, CD25) in T cells.

The discrepancy of results observed between in vitro and in vivo experiments suggest that BMSCs suppress T cell proliferation through a range of different mechanisms, the importance of which perhaps depends on the surrounding microenvironment and cellular milieu at the time of interaction.

Interactions between BMSCs and B Cells

A proportion of both acute and chronic rejection episodes have been attributed to antibody-mediated events (Colvin and Smith, 2005). The documented effects of BMSCs on B cell proliferation, differentiation, and immunoglobulin (Ig) production have been varied and, at times, contradictory. BMSCs were shown to suppress LPS-induced B cell proliferation (at a high BMSC: B cell ratio of 1:2, but not at the lower ratio of 1:10) and differentiation, as well as to inhibit IgM and IgG1 secretion (Asari et al., 2009). An in vivo mouse heart transplant model also demonstrated that BMSCs reduced intragraft IgG as well as circulatory IgM (Ge et al., 2009). The inhibition of Ig synthesis in the in vitro system was demonstrated to proceed via the production of MMPs and CCL2. The authors outline a possible CCL2 (and CCL7) cleavage by the MMPs, converting a typically agonistic CCL2 into an ''antagonistic'' form. It was suggested that this antagonistic CCL2 binds to CCR2 on the B cell, leading to the downregulation of BLIMP-1, which then directly inhibits Ig synthesis (Rafei et al., 2008). A level of specificity was also observed when this system was explored in rOVA-immunized mice. The decrease in B cell BLIMP-1 mRNA in response to coculture with BMSCs has been shown elsewhere (Asari et al., 2009), as has the ability of MMPs to generate truncated CC chemokines with anti-inflammatory properties (McQuibban et al., 2002). Therefore, this model appears to be a highly plausible explanation for BMSC-generated soluble factors that have the ability to regulate B cell Ig production.

BMSC Influence on Dendritic Cells

Through the capture of antigen in the periphery, migration to the draining lymph nodes, and subsequent antigen presentation, dendritic cells (DCs) can efficiently and rapidly activate T and B cells. Both donor and recipient DCs can trigger rejection following transplantation. Donor DCs, following an encounter of inflammatory signals, express a high level of MHC class II molecules that can display intact alloantigen to recipient T cells, thus initiating the direct pathway of allorecognition. The indirect pathway operates when recipient DCs present processed alloantigen (LaRosa et al., 2007; Shoskes and Wood, 1994).

BMSCs have been shown to inhibit differentiation of precursors into DCs, as well as to suppress maturation (a process that involves the upregulation of costimulatory molecules and MHC class II) (Nauta et al., 2006a). Furthermore, DCs that have been cultured with BMSCs are unable to stimulate $CD4^+$ T cell proliferation and produce an augmented cytokine profile (Uccelli et al., 2008). By mediating changes to antigen-presenting cells, BMSCs are clearly in a position to modulate a host of immune responses; however, the precise molecular mechanism responsible for their impact on DC function remains under investigation. For example, it has been shown repeatedly that exposure to BMSCs promotes a ''regulatory'' or ''tolerogenic'' DC phenotype (Li et al., 2008b; Wehner et al., 2009; Zhang et al., 2009). These tolerogenic DCs are maintained in an immature-like state, indicated by the downregulation of CD11c, CD80, CD86, and CD40 and upregulation of CD11b (Zhang et al., 2009). This change in DC phenotype was not reversed by the addition of LPS, suggesting the formation of a relatively stable phenotype. Tolerogenic DCs also produced a lower level of proinflammatory cytokines and a higher level of anti-inflammatory cytokines in comparison with classical mature DCs. Rapidly proliferating T cells were suppressed in the presence of a tolerogenic DC population (Zhang et al., 2009). The ratio of T cell subsets within the overall population also appears to be altered in the presence of this tolerogenic DC population; an increase in the frequency of Tregs has been documented (Ge et al., 2009; Li et al., 2008b).

DC migration from the periphery toward more central T cell areas in the lymph nodes typically occurs following the cell surface upregulation of CCR7 and the concurrent downregulation of E-cadherin. BMSCs inhibited both CCR7 expression (therefore, chemotaxis to CCL19) and also dampened the loss of E-cadherin from activated DCs (English et al., 2008). Should the same changes occur in vivo, their combined effects would maintain DCs in the periphery of the lymph node and, therefore, limit T cell activation.

PGE_2 and the Notch signaling pathway have both been implicated in BMSC-mediated regulation of DCs. DCs have been shown to express the PGE_2 receptors EP2 and EP4, the activation of which led to an inhibition of DC function (Harizi et al., 2003). Upregulation of both the Notch ligand, Jagged-2, and the Notch-2 receptor on the cell surface of tolerogenic DCs has been reported following exposure to BMSCs (Spaggiari et al., 2009; Zhang et al., 2009). The inhibition of DC Jagged-2 expression resulted in the reversal of BMSC effects on T cell proliferation (Zhang et al., 2009). It is possible that lateral Notch activation could be occurring in the tolerogenic DC population.

Interactions between BMSCs and Other Immune Cells

Natural killer (NK) cell involvement can have a significant impact on the outcome of organ transplantation. NK cells identify allogeneic cells either via the absence of self-MHC molecules on the cell surface or by the recognition of stimulatory receptors. In addition to the release of IFN-γ and TNF-α, a potent cytolytic response can be elicited following NK cell activation (LaRosa et al., 2007).

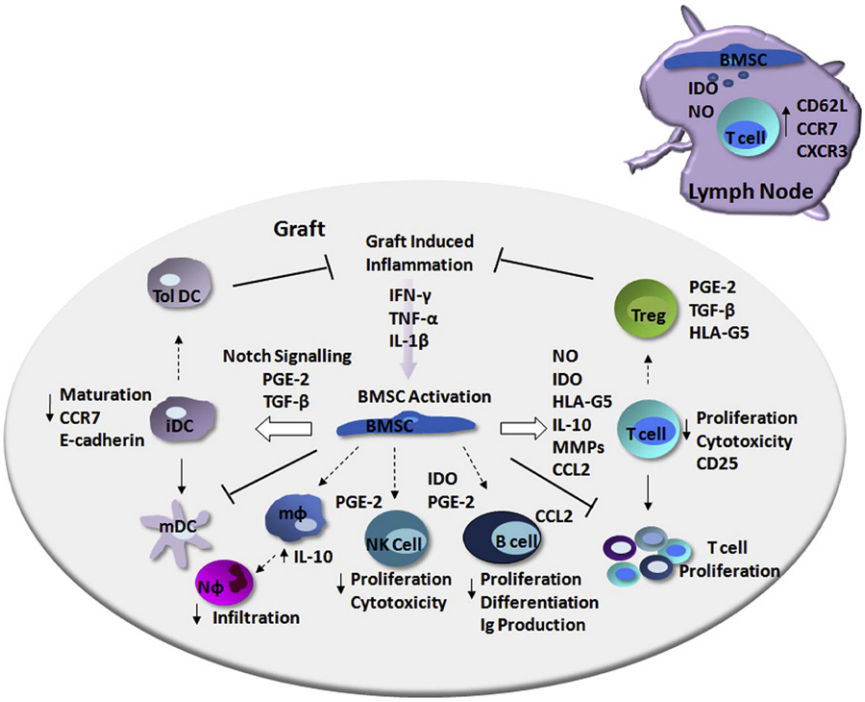

Figure 1. BMSCs Delay Graft Rejection through Multiple Immunomodulatory Mechanisms

BMSCs potentially aid graft acceptance via the generation of Tregs and tolerogenic (Tol) DCs. Recruited BMSCs are activated by proinflammatory cytokines generated through graft-induced inflammation. BMSCs inhibit T cell proliferation, inhibit cytotoxicity, and reduce CD25 surface expression via an array of factors including NO, IDO, HLA-G5, IL-10, MMPs, and CCL2. The Notch signaling pathway, PGE_2, and TGF-β are thought to play an important role in the regulation of DCs. BMSCs also modulate B cells, NK cells, and macrophages through similar mechanisms. Neutrophils are indirectly modulated by BMSCs through macrophage production of IL-10. Furthermore, BMSCs inhibit the migration of DCs to the lymph nodes, thus maintaining alloantigen-loaded APCs in the graft. The enhancement of T cell migration to the secondary lymphoid organs, following the BMSC-induced upregulation of CCR7 and CD62L, generates a system in which stimulating DCs and APCs are effectively compartmentalised. The combination of the generation of regulatory mediators, such as Tregs and tolerogenic DCs, in addition to the prevention of T cell activation (in response to alloantigen presentation) could serve to prevent graft rejection. Mφ, macrophage; Nφ, neutrophil; iDC, immature DC; mDC, mature DC.

BMSCs inhibited cytokine (IL-2, IL-15)-driven proliferation, significantly reduced IFN-γ production, and prevented the upregulation of the activation markers NKp30 and NKG2D on NK cells (Spaggiari et al., 2008). A potential synergistic activity of IDO and PGE_2 has been implicated for the BMSC-mediated inhibition of (IL-2 induced) NK cell proliferation and cytolytic activity (Spaggiari et al., 2008). The impact of BMSCs on other immune cells of the innate response has been poorly characterized.

An abundant neutrophil infiltrate can be found in rejected organs. BMSCs were demonstrated to have an indirect impact on neutrophil migration via the modulation of macrophage cytokine release. BMSCs stimulated the release of IL-10 from tissue-resident macrophages, thus limiting the extent of the neutrophil infiltrate (Németh et al., 2009). In a mouse model of sepsis, it was postulated that TLR4 stimulation combined with increased NO and TNF-α exposure resulted in NF-κB activation and, therefore, an upregulation of COX2 in BMSCs. This sequence of events would increase the availability of substrates for PGE_2 synthesis. PGE_2 released by BMSCs can bind to EP2 and EP4 on the macrophage cell surface; a subsequent increase in cAMP is potentially responsible for the increase in IL-10 (Németh et al., 2009).

In summary, BMSCs modulate the immune response through an array of mechanisms, which will likely aid graft acceptance in the setting of cellular and solid organ transplantation (Figure 1).

Immune Modulation by MSC Derived from Other Tissues

It is also important to reiterate that MSCs have been isolated from a number of tissues in addition to bone marrow, such as adipose tissue, Wharton's jelly, and umbilical cord blood (da Silva Meirelles et al., 2006; Kern et al., 2006; Yoo et al., 2009). While the field has focused on bone-marrow-derived MSCs, evidence that MSCs derived from other tissues also exhibit immunosuppressive properties is beginning to emerge. A small number of comparison studies of both mouse and human BMSCs from different sources suggest measurable differences in the regulatory effects on immune cells (Bochev et al., 2008; Hegyi et al., 2010; Ivanova-Todorova et al., 2009). For example, adipose-derived MSCs had a more potent effect on the inhibition of Ig synthesis and a greater inhibition of DC precursor differentiation (Bochev et al., 2008; Ivanova-Todorova et al., 2009). These observations stress the importance of understanding the mechanism of action of the particular MSC population in question and highlight the need for more exhaustive comparison studies.

BMSCs As a Therapeutic Tool in Cell and Solid Organ Transplantation

Source of BMSCs

BMSCs to be used in combination with cell or organ transplants may come from autologous, donor-derived (allogenic), or third party (allogeneic, derived from neither recipient nor donor) sources. Autologous cells are clearly the safest option for clinical cell therapy in terms of the relative risk of rejection or graft versus host reactions. However, there are circumstances in which healthy autologous, HLA-matched or haploidentical cells will not be available. In these scenarios, an "off the shelf" therapy consisting of third-party allogeneic BMSCs would provide an immediate source ready for clinical utility, and is, therefore, theoretically very attractive. However, there are many important considerations to be taken into account, given the mechanisms by which BMSCs may exert their immunomodulatory functions, as described above.

BMSC Number and Route of Administration

In addition to selecting the appropriate source when considering clinical use of BMSCs, before this population is used in

mainstream cellular and solid organ transplantation, optimal cell numbers and route of administration must be determined. Intravenous administration has been utilized throughout human trials and has also proven to be a suitable route for BMSC infusion in animal models. An additional possibility is to infuse BMSCs into the donor organ prior to transplantation. In a mouse model of diabetes, BMSCs were introduced under the kidney capsule in conjunction with the donor islets, facilitating graft acceptance (Ding et al., 2009). This method could possibly aid the formation of a transplant microenvironment in which BMSCs can exert immunomodulatory effects. Further study is required to elucidate BMSC homing in a transplantation model.

In clinical trials to date, the number of BMSCs infused has ranged from 0.4×10^6 to 10×10^6/per kg of bodyweight (Le Blanc et al., 2008; Macmillan et al., 2009). At this stage, no significant correlation has been made between the dose of BMSCs received and clinical outcome. Furthermore, single, double, and repeated doses of BMSCs have been administered, with no obvious pattern to the outcome observed in each variation of the protocol. For example, some patients responded to a second infusion following a nonresponse to the first, while others failed to respond even after multiple infusions (Le Blanc et al., 2008).

Importantly, no BMSC-related adverse affects have been recorded in clinical trials. In the aforementioned GvHD clinical trial, patient mortality following BMSC infusion was mainly due to opportunistic infection, which was not considered surprising given the patient's status (Le Blanc et al., 2008).

Recently details have emerged from larger-scale, placebo-controlled phase III clinical trials utilizing third-party BMSC as a first and second line therapy to treat GvHD and steroid-resistant GvHD, respectively. A significant placebo effect was observed in these studies, in that BMSC were no more effective than placebo over all. In contrast, BMSC treatment did correlate with a significant improvement in patients with steroid-resistant liver or gastrointestinal GvHD (Mills, 2009). Again, these trials highlight the importance of clarifying how BMSCs exert their effects in vivo and achieving a better understanding of their interaction (s) with existing drug therapies in order to facilitate successful translation to the clinic.

Immunogenicity of Allogeneic BMSC

While BMSCs have been considered to be relatively immune privileged, more recent findings suggest that non-self BMSCs are immunogenic. In particular, under certain conditions, upregulation of both MHC class I and class II on BMSCs has been observed (Chan et al., 2008). A small number of reports provide evidence that both human and mouse BMSCs have the capacity to present antigen and, subsequently, induce effector T cell responses in vitro (François et al., 2009; Romieu-Mourez et al., 2009; Stagg et al., 2006) and memory T cell responses in vivo (Nauta et al., 2006b). Nonetheless, preclinical models and clinical trials using both syngeneic (or autologous) and allogeneic BMSCs have demonstrated no adverse events associated with allogeneic BMSCs (Chen et al., 2009; Le Blanc et al., 2008). Moreover, data from a mouse model examining efficacy of syngeneic and allogeneic BMSCs in wound repair indicate that syngeneic and allogeneic BMSCs do not evoke an immune response unlike allogeneic fibroblasts (Chen et al., 2009). Thus, the degree to which allogeneic BMSCs may induce immune responses in vivo remains unclear.

Data from a clinical trials utilizing BMSC for treatment of acute, steroid-resistant GvHD also indicates that the administration of allogeneic BMSCs does not appear to trigger an immune response. In this study, patients were given BMSCs from HLA-identical, haploidentical, or third-party donors. Out of a total of 55 patients, 27 exhibited a ''complete response'' following one dose of BMSCs; of this group, 2 patients received HLA-identical BMSCs, 3 haploidentical, and 24 third party (Le Blanc et al., 2008). This finding indicates that the administration of allogeneic BMSCs does not significantly impact on the therapeutic outcome, at least in the setting of GvHD. Indeed, one might also speculate that BMSCs with an allogeneic origin might offer a more beneficial clinical outcome and, if so, could perhaps shed light into the mechanism by which the BMSCs impact the immune response in a therapeutic setting. There are potentially a number of significant advantages to be gained if third-party BMSCs can be utilized. First, BMSCs can be generated in bulk, providing a more economical option and potentially a more uniform source of donor cells. In addition, the use of third-party BMSCs enables cells to be available on demand so that treatment does not have to be delayed during a period of cell culture and characterization. This factor becomes even more relevant in the case of organ availability for transplantation from deceased donors.

Taking all of the available evidence into consideration, it seems likely that the potential for BMSCs to act as APCs and, therefore, become immunostimulatory is limited to a specific combination of conditions. However, the clinical significance of this possibility is clearly an important issue which warrants further investigation in order to define the specific conditions that could lead to a deleterious outcome.

Effects of Immunosuppressive Drugs on BMSC Function

As all transplant patients receive immunosuppressive drugs, it would be important to fully understand the extent to which these drugs may impact on BMSC function. A number of in vivo studies have explored the use of BMSCs in combination with conventional immunosuppression. In a mouse allogeneic heart model, BMSCs and rapamycin coordinated to yield a synergistic effect and induced tolerance as measured by the absence of rejection up to 100 days posttransplantation and subsequent acceptance of donor-derived skin graft (Ge et al., 2009). The specificity of tolerance was demonstrated by showing that a third-party skin graft was not accepted by the treated recipients. However, when a mixed lymphocyte reaction was performed, the suppression of PBMC proliferation by rapamycin and tacrolimus was adversely affected by the presence of human BMSCs. Yet, in a separate system, BMSCs complemented the inhibition of PBMC proliferation by mycophenolic acid (MPA) (Hoogduijn et al., 2008). In both swine and rat models, the administration of cyclosporine A (CsA) with BMSCs resulted in prolongation of a composite tissue and skin graft, respectively (Kuo et al., 2009; Sbano et al., 2008). This synergy between BMSCs and MPA with regard to suppression of T cell proliferation could be due to distinct mechanisms of action employed by BMSCs and MPA. Therefore, MPA might be the drug of choice in combination therapy. It is likely that immunosuppressive drugs will be required initially (following transplantation) to allow BMSC survival and to create an environment in which BMSCs can become activated in order to achieve their effect. Even if BMSCs

Table 1. Preclinical Models Utilizing MSC Immune Modulation and Repair Capacity

Disease model	Animal Model	MSC Source	Conventional Immunosuppression	Outcome	References
Heart transplantation (semi-allogeneic)	mouse	donor bone marrow	no	long-term graft survival	(Casiraghi et al., 2008)
Skin transplantation (allogeneic)	rat	donor bone marrow	CsA, certain groups	MSC + CSA, graft survival prolongation; MSC alone, accelerated rejection	(Sbano et al., 2008)
Islet transplantation (allogeneic)	mouse	syngeneic bone marrow	no	long-term graft survival	(Ding et al., 2009)
Skin transplantation (allogeneic)	baboon	donor bone marrow	no	prolongation of donor and third party skin grafts	(Bartholomew et al., 2002)
GvHD	mouse	syngeneic bone marrow	no	prevention of disease	(Ren et al., 2008)
Composite tissue transplantation (allogeneic)	swine	donor bone marrow	CsA, certain groups	MSC alone, prolonged graft survival; MSC + Irr + BMT + CsA, significantly prolonged graft survival	(Kuo et al., 2009)
GvHD	mouse	donor bone marrow	no	prevention of GvHD; treatment of established GvHD	(Polchert et al., 2008)
Experimental autoimmune encephalomyelitis	mouse	allogeneic bone marrow	no	ameliorate disease	(Rafei et al., 2009)
Sepsis	mouse	syngeneic bone marrow	no	ameliorate disease	(Németh et al., 2009)
Ischemic acute renal failure	rat	syngeneic bone marrow	no	improved renal function	(Tögel et al., 2005)
Osteoarthritis	goat	syngeneic bone marrow	no	regeneration of meniscal tissue	(Murphy et al., 2003)
Critical-size bone defect	dog	allogeneic bone marrow	no	enhanced repair, no adverse immune response	(Arinzeh et al., 2003)
Critical-size bone defect	rabbit	allogeneic peripheral blood and bone marrow	no	enhanced repair, no adverse immune response	(Wan et al., 2006)
Heart transplantation (allogeneic)	mouse	recipient/donor/third party bone marrow	rapamycin	long term graft survival	(Ge et al., 2009)
Myocardial infarction	rat	allogeneic bone marrow	no	improved global Lv function, no evidence of inflammatory response	(Dai et al., 2005)

Summary table detailing the outcomes of animal models utilizing MSC immune modulation and repair capacities. CsA, cyclosporine A; GvHD, graft-versus-host disease; Irr, Irradiation, BMT, bone marrow transplant; Lv, left ventricular.

are not capable of enabling drug-free graft survival or tolerance, their use could provide significant benefit in transplantation models by reducing the overall load of immunosuppressive drug therapy required to maintain long-term graft function.

In a number of animal models, BMSCs have been infused without accompanying immunosuppression. For example, pancreatic islets transplanted to an immunocompromised mouse were not rejected when introduced in combination with BMSCs, as demonstrated by long-term normoglycaemia (Ding et al., 2009). Monotherapy by infusion of BMSCs alone also delayed graft rejection in an immunocompetent semiallogeneic heart model (Casiraghi et al., 2008). However, at least one report has shown that BMSC administration alone has resulted in the acceleration of graft rejection in an animal model (Sbano et al., 2008). Such undesirable outcomes highlight the necessity for a better overall understanding of the system in question to ensure that the use of BMSCs and adjunctive therapy can be tailored effectively.

Nevertheless, data from animal studies (Table 1) and clinical experience with MSCs (Table 2) suggest that modulation of the immune response and the induction of tolerance can potentially be achieved via the administration of MSCs (the lattermost realistically following a brief period of immunosuppressive drug administration), but a more conclusive interpretation remains to be determined following long-term observation of treated patients.

Trials that use MSC in solid organ transplantation (kidney) are currently in their infancy (www.clinicaltrials.gov), and there are over 100 clinical trials utilizing the immunomodulatory and proreparative effects of MSC. The results of these trials will undoubtedly provide further insight into the application of therapeutically administered MSC in transplantation.

Conclusion and Future Prospective

Overall, the ability of BMSCs to mediate inhibition of T cell proliferation, DC maturation and migration, B cell Ig synthesis, and NK function indicates that these cells have the capacity to subdue the immune response. Furthermore, the generation of tolerogenic DCs and Tregs represents a viable physiological mechanism by which sufficient immunomodulation could occur in order to offer clinical benefit.

One point that is clear is that BMSCs appear to require activation in order to develop their full immunomodulatory potential,

Table 2. Clinical Experience of MSC in Immune modulation

Disease	Patients (N)	MSC source	Conventional Immunosuppression	Outcome	References
Acute GvHD (GI tract and liver)	55	HLA-identical, haploidentical, or mismatched bone marrow	yes	30 complete response	(Le Blanc et al., 2008)
Acute GvHD	32	allogeneic bone marrow	yes	77% of patients responded	(Kebriaei et al., 2009)
Acute GvHD	192	allogeneic bone marrow (prochymal)	yes	no significant difference from placebo overall	(Mills, 2009)
Acute GvHD steroid-resistant	260	allogeneic bone marrow (prochymal)	yes	significant improvements in patients with gastrointestinal and liver GvHD	(Mills, 2009)
Multiple sclerosis	10	allogeneic bone marrow	-	variable responses	(Mohyeddin Bonab et al., 2007)
Scleroderma	1	allogeneic bone marrow	-	improved	(Christopeit et al., 2008)

Summary table detailing the patient number, MSC source, use of immunosuppression, and outcome of clinical trials with MSCs. GI, gastrointestinal.

where signals produced by various immune cells act on the BMSCs to effect a change and induce subsequent release of mediators. These mediators then regulate the initiating or surrounding cells, "closing the loop," so to speak, on the cycle of BMSC-mediated immune modulation. It may well be due to this cycle of indirect events that so many conflicting findings are present in the experimental literature. Indeed, a discrepancy is often reported for in vitro assays with regard to whether BMSCs regulate the activity of other cell populations via cell-cell contact or soluble factors. One explanation is that soluble factors drive chemotaxis of the immune cell to within close proximity of the BMSCs. Cell-cell contact or diffuse mediators within the microenvironment can subsequently enhance suppression or immunomodulation. It is highly plausible that such a requirement for recruitment exists in vivo, yet these scenarios are understandably difficult to replicate outside of a live recipient.

Key mechanistic differences have been observed between human and mouse BMSCs, which underscores that caution should be exercised when attempting to translate results from mouse in vivo models to understand clinical events. Nonetheless, mouse models remain an extremely useful experimental tool. A number of contradictory findings resulting from both in vitro and in vivo animal models can perhaps be explained by the heterogeneity of cellular populations deemed to be "BMSCs." Indisputably, the field would benefit from clear and defined descriptions of populations used in each study, as well as the ability to prospectively isolate relatively pure MSC populations.

Theoretically, BMSCs have the potential to enhance cancer progression as they have the ability to generate an environment in which the immune response is suppressed (and, therefore, also the immune response to the tumor). Although BMSCs proliferate vigorously in vitro, little evidence of significant in vivo cell division has been reported. The engraftment of infused BMSCs also appears to be limited. On the other hand, BMSCs secrete a plethora of growth factors, cytokines, and MMPs, including VEGF and IL-6, that could potentially drive tumor growth. VEGF is known to drive angiogenesis, a requirement for tumor growth and subsequent metastasis (Kögler et al., 2005). Further evidence demonstrates that BMSCs are recruited to sites of neoplasia, where they integrate into the tumor-associated stroma (Spaeth et al., 2009). Consequently, further investigation with regard to the potential adverse effects of BMSC infusion must be undertaken.

As discussed above, in vivo models have shown that BMSCs can be used in combination with a number of immunosuppressive drugs currently utilized in the clinic. The implementation of a short-term regimen of conventional immunosuppression alongside BMSC infusion looks promising and has the potential to induce tolerance in the recipient (Ge et al., 2009). The safety and efficacy of BMSC administration looks encouraging, yet long-term patient observation must be undertaken to ensure that no significant adverse affects are caused by infusion of in vitro cultured BMSCs (Le Blanc et al., 2008).

Data that emerges from early clinical trials that use BMSCs in cellular or solid organ transplantation settings will undoubtedly offer important insights for the field. However, optimization of the regimen implemented, cell number, and route of administration must be undertaken if the full potential of BMSCs is to be realized. The complexity of the potential interactions between MSCs and the variety of immune cell mediators clearly points to the importance of additional in vivo studies, in a range of experimental models, in order to establish the role of BMSCs as well as to further elucidate the immunoregulatory mechanisms at play in different clinical situations.

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